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Original Article

Noninvasive prenatal testing for fetal trisomy in a mixed risk factors pregnancy population

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ABSTRACT

Objective: This study assesses the performance of noninvasive prenatal testing (NIPT) for fetal aneuploidies in a mixed risk factors pregnancy population.
Materials and methods: Data review of 169 pregnant women undergoing prenatal aneuploidy screening in a single tertiary medical center was conducted. Indications included maternal anxiety, advanced maternal age, abnormal nuchal translucency, and high/moderate risk of first trimester Down syndrome screening. Multifetal pregnancies and patients receiving *in vitro* fertilization were also enrolled for analysis.
Results: A total of 169 patients were enrolled in this study during a time period from July 2012 to June 2014. For patients' $\geq$ 34 years, anxiety about amniocentesis was the most common reason for patients selecting NIPT for fetal aneuploidy screening, with 107 (88.4%) patients choosing NIPT for this reason. Among the total patient population, two patients showed a positive result from NIPT. One patient displayed 47, XXY, which was confirmed to be a false-positive result. The other patient displayed trisomy 18, which was confirmed by an amniotic cell culture. The sensitivity for NIPT is 100% with the specificity 99.4%.
Conclusions: NIPT for fetal aneuploidy in a mixed risk factors pregnancy population showed high accuracy. NIPT applied to the low risk population might reassure the anxious family.

Introduction

Prenatal screening for fetal aneuploidy is a standard offering in most parts of the world. Fetal aneuploidies, such as trisomy 21 (T21, Down syndrome), trisomy 18 (T18, Edwards syndrome), and trisomy 13 (T13, Patau syndrome), as well as aneuploidies related to the X and Y chromosomes are the most common chromosomal abnormalities. Using the first trimester screening (FTS), consisting of maternal serum markers and nuchal translucency (NT) measurement, we were able to identify 85–95% of T21 and T18 cases, with a 5% rate of false positives. Invasive procedures, amniocentesis and chorionic villus sampling were taken as diagnostic tools [1,2]. The discovery of the presence of fetal cell-free DNA (cfDNA) and RNA in maternal plasma, combined with new DNA sequencing technology, has allowed noninvasive prenatal testing (NIPT) of common fetal trisomy with high sensitivity and specificity. From a multicenter prospective cohort study, NIPT provided detection rates > 99% for T21 and false-positive rates < 0.1% [3].

Due to the high accuracy of this new technology, the International Society for Prenatal Diagnosis, the National Society of Genetic Counselors, the American College of Obstetricians and Gynecologists (ACOG), and the Society for Maternal–Fetal Medicine (SMFM) have published committee opinions stating that cfDNA testing could be offered to pregnant women at high risk for fetal aneuploidy as a screening option after counseling [4–6]. Therefore, this technology might reduce the number of unnecessary invasive procedures, compared with conventional maternal serum

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Table 1
Maternal characteristics and gestational age of blood sampling.

Maternal age	
Median (y):	35.31
Advanced maternal age (≥ 34 y old):	121 (71.6%)
≤ 28 y old (*n*, %):	4 (2.5%)
29–33 y old (*n*, %):	44 (26.2%)
34–38 y old (*n*, %):	90 (53.3%)
39–42 y old (*n*, %):	27 (15.9%)
≥ 43 y old (*n*, %):	4 (2.5%)
Gestational age at blood sampling	
Median (wk)	13.45 wk
Range (wk)	7–31 wk
6–8 wk (*n*, %)	3 (1.8%)
9–12 wk (*n*, %)	64 (39.3%)
13–16 wk (*n*, %)	74 (45.5%)
17–20 wk (*n*, %)	17 (10.4%)
21–24 wk (*n*, %)	3 (1.8%)
25–28 wk (*n*, %)	1 (0.6%)
>28 wk (*n*, %)	1 (0.6%)
History or family history of aneuploidies (*n*, %)	**4 (2.4%)**

aneuploidy screening. Nevertheless, the power of this new technology in a mixed risk factors group has not yet been fully assessed. This study reports the finding from an observational study which was carried out to assess the performance of NIPT for fetal aneuploidy in a mixed risk factors pregnancy population.

Materials and methods

From July 2012 to June 2014, data were collected from a total of 169 pregnant women undergoing NIPT in a single tertiary medical center. Indications included maternal anxiety, advanced maternal age (≥ 34 years), abnormal NT, high/intermediate risk of maternal serum screening (2 and 4 markers), and high/intermediate risk result from first trimester screening. Twin pregnancies (total number 12), triplets (total number one), and patients receiving artificial reproductive technology (ART) to conceive, were also enrolled for analysis.

Result

A total of 169 pregnant women from a single tertiary medical center were recruited in this study. Maternal age ranged from 27 to 44 years. Median age was 35.31 years and 71.6% (121/169) were ≥ 34 years (Table 1). Thirty-one pregnant women had received ART, including six with intrauterine insemination, 24 with *in vitro* fertilization (IVF), and one with intracytoplasmic sperm injection (ICSI) (Table 2). Four women had a history or family history of aneuploidies.

Indication for NIPT

For patients < 34 years of age (48 patients) 27 (56.3%) asked for NIPT due to anxious feelings of possible fetal aneuploidies. Other indications included high risk of T13, T18, or T21, which accounted for 10 patients (20.8%), and intermediate risk of T13, T18, or T21, which accounted for another 10 patients (20.8%) (Fig. 1). For patients ≥ 34 years, anxiety about amniocentesis was the most common reason for the patient selecting NIPT for fetal aneuploidy screening, with 107 (88.4%) patients choosing NIPT for this reason. Other indications included 7 (5.8%) patients of high risk of T13, T18, or T21 and 7 (5.8%) patients of intermediate risk of T13, T18, or T21 (Fig. 2). For the 107 patients who were anxious about amniocentesis, 24 (22.4%) had taken NIPT as a primary test and had neither received FTS nor maternal serum screening (2 markers and 4 markers); 35 (32.7%) patients had taken NIPT before FTS; and 47 (43.9%) patients had taken NIPT and FTS on the same day.

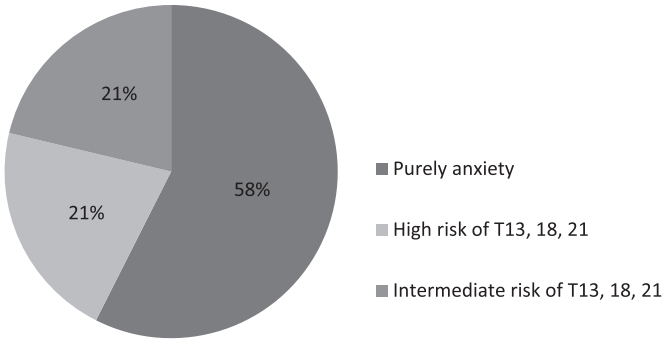

Fig. 1. Indication of NIPT—patients less than 34 years old.

Fetal aneuploidies

Among 169 patients, two patients showed positive results from NIPT. One showed positive for 47, XXY and the other showed positive for T18. Both later received amniocentesis for diagnostic confirmation. The patient who had the result of 47, XXY was later shown to have a false-positive result. The fetus was confirmed to be 46, XY using amniocentesis and was shown to be a healthy male baby after delivery. The other patient, who showed positive for T18 from NIPT, went on to have amniocentesis which confirmed the diagnosis of T18. After diagnosis, the patient decided to terminate the pregnancy.

The sensitivity for fetal aneuploidy screening in this study was 100% with a specificity of 99.4%.

Discussion

Since Lo et al [7] discovered the presence of fetal DNA fragments in maternal serum, a new technology for prenatal aneuploidy

Table 2
The ways of patients got conceived.

Method of conception	Number of patients	Percent (%)
IUI	6	3.6
IVF	24	14.2
IVF (ICSI)	1	0.6
Nature	138	81.7
Total	169	100.0

ICSI = intracytoplasmic sperm injection; IUI = intrauterine insemination; IVF = *in vitro* fertilization.

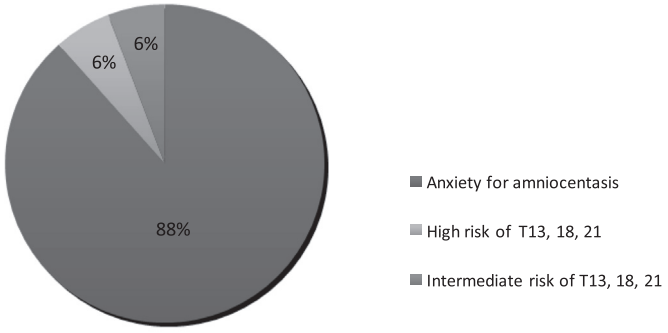

Fig. 2. Indication of NIPT—patients ≧ 34 years old.

screening has emerged. For fetal maternal specialists, how to apply NIPT and what group of patients should be offered NIPT is still uncertain.

Low risk group

This study examines data from a single tertiary medical center where NIPT has been provided to patients since July 2012. Due to the health insurance policy in Taiwan, prenatal aneuploidy screening for patients < 34 years is not covered by national health insurance. This means any screening in this age group is done under self-payment. Some patients ($n = 27$, 16% of total patients receiving NIPT) were concerned with the accuracy of the maternal serum test or FTS and opted to have a more accurate test. Despite it being more expensive, they asked for NIPT. This practice is not recommended according to the ACOG announcement in 2012. NIPT is only recommended for patients in a high risk group [6]. NIPT application to the low risk group is still a matter of conflict; a study published in 2014 reported that 1914 women of general risk in the obstetric population showed significantly lower false-positive rates and higher positive predictive values for detection of T21 and T18 When the physicians applied NIPT rather than traditional screening [8].

Advanced maternal age group

For women > 34 years, national health insurance in Taiwan covers part of the prenatal testing payment and makes amniocentesis much cheaper than NIPT, which must be paid fully by the patient. Amniocentesis costs 8000 New Taiwan Dollars (NT) (265 US dollars), and NIPT costs around 24,000 NT (795 US dollars). Nevertheless, 107 patients > 34 years selected NIPT for fetal aneuploidy screening due to anxious feelings about amniocentesis. Amniocentesis tests are highly accurate; however, they are associated with an iatrogenic miscarriage rate up to 1% [9]. As women of advanced age find it more difficult to get pregnant, they are more concerned with the potential risk of invasive procedures.

Multifetal pregnancies

The current study did not exclude multiple gestations; there were 12 twin pregnancies and one set of triplets. The results of NIPT were all negative and confirmed to be correct after birth. Although the power of NIPT in multifetal pregnancies has not yet been established, there have been several studies showing promising results [10–12]. The main problem for multifetal pregnancies is due to the reporting rate of results being lower than in singleton pregnancies due to a lower fetal fraction and also from the result not being able to determine which baby is affected.

ART group

Patients receiving ART to get pregnant were a special group of patients who asked for NIPT. As many of these patients face the problem of infertility and were paying a lot of money to get pregnant, they were keen to have a more accurate screening test, despite the expense. In the current study, patients receiving ART accounted for 18.3% ($n = 31$) of all patients. Screening tests for genetic problems for patients receiving IVF do not need to be NIPT because preimplantation genetic screening or preimplantation genetic diagnosis (PGS/PGD) are promising tools that can give results before getting pregnant. Methods may involve biopsy polar bodies, blastomeres or trophoblast [13]. If patients have PGS/PGD, the need for NIPT might disappear.

The accuracy of NIPT

A sensitivity of NIPT of 100% with a specificity of 99.4% was observed in this study. Two positive results were identified through NIPT. One result was shown to be a false-positive result of 47, XXY. The other result was a true-positive result of T18. Both patients received amniocentesis for diagnosis. NIPT uses mathematical calculation to "estimate the amount" of each chromosome. Although numerous studies have already proven it more accurate than conventional screening, it still has some restrictions. For example, a fetal amount of at least 4% is necessary for an accurate result; a lower level can cause false-negative results [3,14,15], false-positive results can also occur. A low false-positive rate of < 1% was observed in other studies [16–19]. Although quite low, this still might cause unnecessary worries to the mother, or even a mistaken decision to have an abortion. Therefore, the ACOG and SMFM announcement in 2012 suggests positive results of NIPT should be followed by an invasive procedure to confirm the diagnosis, which we also agree is necessary [6].

Conclusion

In conclusion, this study shows that NIPT had better performance than conventional screening methods in the mixed risk patient population study group. The use of NIPT in the low risk population is feasible and might turn into a primary screening method for fetal aneuploidy in the future. NIPT for multifetal pregnancy can be considered as a choice for prenatal screening; however, some limitations must be taken seriously and physicians should have prenatal counseling with the patient before and after the screening. Due to the small population of this study, further investigation is needed.

Conflicts of interest

All the authors declare no conflict of interest.

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